*Plant Pathology* (2005) **54**, 431–440 Doi: 10.1111/j.1365-3059.2005.01241.x

# *Tomato spotted wilt virus* in peanut tissue types and physiological effects related to disease incidence and severity*

D. Rowland[a†‡], J. Dorner[a], R. Sorensen[a], J. P. Beasley Jr and J. Todd[b]

[a]*USDA-ARS, National Peanut Research Laboratory, 1011 Forrester Dr. SE, Dawson, GA 39842; and* [b]*University of Georgia, PO Box 1209, Tifton, GA, 31793, USA*

Three peanut cultivars, Georgia Green, NC-V11, and ANorden, were grown using production practices that encouraged the development of *Tomato spotted wilt virus* (TSWV). The progression of TSWV infection was examined through the season using enzyme-linked immunosorbent assay (ELISA) tests on different tissue types [roots, leaves, pegs (pod attachment stem structures) and pods] and the effect of TSWV infection on physiological functions was examined at three harvest dates. Plants were classed into three severity categories: (i) no TSWV symptoms or previous positive ELISA tests; (ii) less than 50% of leaf tissue exhibiting TSWV symptoms; and (iii) greater than 50% of leaf tissue affected. TSWV showed a slow rate of infection at the beginning of the season and a greater percentage of infection of the roots than in the leaves. Photosynthesis was reduced in virus-affected infected plants by an average of 30% at the mid-season harvest and 51% at the late season harvest compared with virus-free plants across all three cultivars. Leaf tissue with symptoms had lower photosynthetic rates than healthy leaves. There were small differences among cultivars, with cv. ANorden maintaining higher average photosynthetic levels than cv. Georgia Green and higher transpirational levels than cv. NC-V11. The ability to maintain high assimilation physiology in the presence of the virus may help cultivars withstand TSWV infection and maintain final yields.

*Keywords*: *Arachis hypogaea*, ELISA, gas exchange analysis, photosynthesis, transpiration, tolerance, water-use efficiency

## Introduction

Virus infection is known to affect plant physiology dramatically, including decreased photosynthesis, increased respiration and altered carbohydrate levels (Shalitin & Wolf, 2000; Ryšlavá *et al*., 2003). The alteration of these physiological processes caused by viral diseases is one of the primary causes of decreased crop productivity across the world (Agrios, 1997). Much of the research concerning plant viruses, however, has been focused on determining the structure, genetics, transport and localization of viruses in plant tissues, with much less effort aimed at understanding the effect of viral infection on host plant physiology (Zaitlin & Hull, 1987; Balachandran *et al*., 1997; Arias *et al*., 2003). Furthermore, viral treatments are extremely difficult to control experimentally, and their physiological consequences can be highly variable, thereby leading to a continued lack of understanding of these effects (Ayres, 1992; Balachandran *et al*., 1997).

There is a paucity of information related to the physiological effects of *Tomato spotted wilt virus* (TSWV) on peanut (*Arachis hypogaea*), even though the virus is known to have devastating effects on horticultural and crop production worldwide (Goldbach & Peters, 1994). TSWV costs an estimated US$100 million annually to southeastern USA agricultural commodities, including the peanut industry, through reduction in yields (Riley, 2004) and has severely impacted on production in almost every US state producing peanuts (Culbreath *et al*., 2000). The virus was initially described by Brittlebank (1919) and has a host range of at least 800 plant species (Lyerly *et al*., 2002). The disease was initially found in peanut in Texas, USA, in the 1970s (Stewart *et al*., 1989) and has now spread to all peanut-producing US states. Symptoms of TSWV infection range from severe stunting of the plant

*Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

†To whom correspondence should be addressed.

‡E-mail: drowland@nprl.usda.gov

*Accepted 21 February 2005*

and vines, discoloured hulls and kernels, dwarfed pod size, elaborate concentric ring spots on leaves, curling, disfigurement and stunting of leaves, decreased pod number and size, and often death of the plant (Culbreath *et al*., 1992a). The first symptoms usually appear a few weeks after planting, and newly virus-affected plants appear throughout the rest of the growing season (Culbreath *et al*., 1992a). It is known that two species of thrips are the primary vectors for TSWV in peanuts: *Frankliniella fusca* and *F. occidentalis* (Todd *et al*., 1995; Armstrong *et al*., 2001). In general, the use of insecticides alone to control thrips has been an ineffective means of suppressing TSWV and it has often been found that the severity of infection is independent of the thrips population (Culbreath *et al*., 1992b, 1999). These results provide evidence of a possible physiological mechanism for TSWV resistance in peanut unrelated to thrips control.

As with most traits, TSWV resistance varies between peanut cultivars, and so too may the associated physiological responses. Although some moderate resistance has been found, there are no known peanut cultivars with complete resistance (Culbreath *et al*., 1999; Lyerly *et al*., 2002). Evidence exists that differences among peanut cultivars in severity of TSWV symptoms are not related to differential responses to the thrips vector, but result from differential tolerance of cultivars to TSWV (Culbreath *et al*., 2000; Garcia *et al*., 2000; Wells *et al*., 2002). This tolerance may be partially physiologically mediated. However, the screening of cultivars for resistance to thrips remains the prevalent method for ranking genotypes for TSWV resistance (Culbreath *et al*., 1999). Because of the inability of cultural and chemical means to affect the severity of TSWV outbreaks, cultivars that suppress epidemics of TSWV remain the most important tools in minimizing yield loss in peanut production (Culbreath *et al*., 1999; Mandal *et al*., 2002; Wells *et al*., 2002), and cultivar selection is important for growers' ability to minimize the effect of TSWV (Culbreath *et al*., 2000).

The objectives of this study were: (i) to follow the progression of infection through different peanut tissue types through the growing season and determine relative differences in disease incidence and severity; (ii) to determine the physiological effects of TSWV infection through time in leaf tissue with and without symptoms; and (iii) to establish whether differences between peanut cultivars in physiological properties relate to viral infection.

## Materials and methods

### Planting and harvest

Peanut cultivars were hand-planted on 18 April 2003 in plots located at the USDA-ARS, National Peanut Research Laboratory in Dawson, GA, USA. Just prior to planting, the field area was fertilized with N:P:K 10–10–10 at a rate of 672 kg $ha^{-1}$ and lime (commercial grade $CaCO_3$) was applied at 1121 kg $ha^{-1}$. Peanut seeds were planted in rows spaced 0·9 m apart and 24·4 m long, with the distance between seeds within a row being 10·2 cm. The early planting date and large interplant distance were chosen to maximize the incidence and severity of TSWV (Culbreath *et al*., 1999). The plot area consisted of a total of 13 rows with nine test rows and two outside border rows on each side of the plot area. Three commonly grown US peanut cultivars were planted in three rows each: Georgia Green, NC-V11 and ANorden. Furrows were treated with Thimet™ · 20G (phorate, BASF Corporation) at a rate of 7·3 kg $ha^{-1}$ just prior to planting in order to control excessive thrips damage early in the season. Plants were irrigated adequately to avoid any symptoms of water stress from planting to 28 July 2003. Pesticides were applied according to the recommended management practices for peanut including weed control, a 14-day fungicide spray schedule to prevent fungal pathogens, and insecticides when pests were present. For a related study testing the relationship between TSWV and aflatoxin contamination in these three peanut cultivars (data not presented), a late-season drought was imposed on 28 July 2003 by eliminating supplemental irrigation and covering the entire plot area (approximately $9{\cdot}1 \times 24{\cdot}4$ m) with a plastic greenhouse to prevent the plots from receiving rainfall. This treatment was continued until harvest.

ELISA testing, TSWV ImmunoStrip ELISA tests (Agdia) were used on peanut tissue according to manufacturer's directions. Briefly, between 0·15 and 0·20 g of tissue was placed in sample extract bags containing Sample Extract Buffer #1 solution. The tissue was placed between two pieces of mesh screen, and by pounding the tissue with a small mallet on the outside of the sample bag, tissue was emulsified into the buffer and test strips were inserted into the solution. For testing kernels, it was important to emulsify the entire pod for testing because TSWV is known to accumulate preferentially in hull tissue (Pappu *et al*., 1999). During testing, the control and test lines developed in 20–30 min and the results of the test could be recorded. Root tissue was washed prior to testing. Leaf, peg (the stem tissue that attaches the underground pod to the above-ground lateral stem of the plant), root and pod (hull and kernels emulsified together) were tested using the above method.

On 12 May 2003, 25 days after planting (DAP), 50 plants of each cultivar were permanently tagged with metal tags on two of the three rows for a given cultivar, using the third row as a border between cultivars. On 12 May 2003 (25 DAP), leaf tissue was collected from the 50 tagged plants of each cultivar and tested for the presence of TSWV. Visible symptoms of TSWV began appearing by 30 May 2003 (43 DAP), so on both 30 May and 27 June 2003 (70 DAP), leaves with and without symptoms (if both were present on a plant) were collected from the 150 tagged plants and tested again for TSWV. Because root tissue could not be obtained from tagged plants on both 12 May (25 DAP) and 30 May (43 DAP) without injury or death, another set of 20 random plants of each cultivar was destructively harvested on these two dates and root and leaf tissue was tested for the presence of TSWV.

On 21 July 2003 (92 DAP), in order to track the progression of the virus more effectively, a subset of tagged plants was chosen for further measurements and categorized according to the severity of their TSWV visual symptoms and previous testing history. Category 1 plants were those that had no previous positive TSWV test (on any tissue type) and had no visual TSWV symptoms; category 2 plants were those with a previous positive TSWV test but had less than 50% of their foliage exhibiting visual TSWV symptoms; and category 3 were plants with a previous positive TSWV test and greater than 50% of their foliage exhibiting visual TSWV symptoms. Five plants in each category within each of the three cultivars (a total of 45 plants) were chosen for further TSWV testing and other physiological testing.

### Gas exchange and physiological measurements

Physiological measurements began on 25 June 2003 (68 DAP). On this date, six tagged plants per cultivar were tested for gas exchange alone, including photosynthesis, transpiration and water-use efficiency. Water-use efficiency was calculated as the ratio of photosynthesis to transpiration (Ehleringer & Osmond, 1989). Gas exchange was measured on the second nodal, apex leaf with an ADC LCi infrared gas analyser (The Analytical Development Company Ltd, UK) between the hours of 08·00 and 13·00 when photosynthetic levels were maximal (data not shown). The second and third physiological harvests occurred on 21 July (92 DAP) and 11 August 2003 (116 DAP), respectively. On these dates, physiological measurements included gas exchange, SPAD chlorophyll content, relative water content (RWC), infrared thermal (IRT) surface temperature and leaf area. Gas exchange, IRT temperature, SPAD chlorophyll content, leaf area and specific leaf area (SLA) were measured on the same second nodal, apex leaf. Leaf surface IRT temperature was measured with an infrared thermometer (Fluke Corporation) just prior to enclosing the leaf in the gas exchange chamber. After gas exchange measurement, the leaf was excised, measured with the model SPAD-502 chlorophyll meter (Soil-Plant Analyses Development Unit) and taken back to the laboratory for further processing. Relative water content was measured by taking an immediate fresh leaf weight, floating the leaf in distilled water under a growth light for 3 h, and then weighing again for turgid weight. The leaf was then dried at 60°C for 72 h and weighed again for dry weight. Relative water content was calculated as follows:

$$\text{RWC} = [(\text{fresh weight} - \text{dry weight})/(\text{turgid weight} - \text{dry weight})] \times 100$$

and expressed as a percentage. Specific leaf area was calculated by dividing leaf area by dry mass.

### Harvest

Plants were harvested on 16 September 2003 (152 DAP) by hand-digging and roots from 20 plants from each row of each cultivar (60 plants per cultivar for a total of 180 plants) were tested for TSWV. The remaining plants within the plot area were left to dry in the plots for approximately 3 days. Pods were removed from plants using an automated thresher (Kingaroy Engineering Works Pty Ltd) and then dried to 7% moisture before weighing for yield. Yield was calculated as kg dry weight per ha of plot area.

### Statistical analyses

Statistical analyses were performed using SAS JMP (SAS, 1997). Logistic regression was used to determine if TSWV infection percentages were significantly affected by cultivar, plant category and leaf type (for leaf analyses only) in different tissue types and at different harvest dates, and Wald chi-squared values were determined. Factorial analysis of variance (ANOVA) was conducted on gas exchange traits and other physiological traits measured examining the effect of cultivar, plant category and leaf type at three harvest dates. Transpiration and water-use efficiency for the first harvest were both log-transformed prior to analysis to satisfy the normality assumption of ANOVA.

## Results

### Tissue infection

Incidence of TSWV disease in leaves was followed from early plant development to mid-season establishment [12 May (25 DAP) to 27 June (70 DAP)] in the full set of tagged plants ($n$ = 150; 50 plants per cultivar). There were no significant differences among cultivars for incidence of TSWV in leaves over the three dates measured (d.f. = 2, Wald chi-squared = 3·78, $P$-value = 0·1514), but there was a greater percentage of leaves with symptoms than without symptoms (d.f. = 1, Wald chi-squared = 12·62, $P$-value = 0·0004), and overall disease increased significantly over time (d.f. = 2, Wald chi-squared = 7·57, $P$-value = 0·0227) (Fig. 1a). Root infection was followed throughout the season from 12 May (25 DAP) to 16 September (152 DAP). Incidence of disease was much higher in roots than in leaf tissue. Similar to the leaves, roots showed no significant difference among cultivars for presence of TSWV across all dates (d.f. = 2, Wald chi-squared = 5·25, $P$-value = 0·0723), but incidence of virus infection increased with time (d.f. = 2, Wald chi-squared = 44·98, $P$-value = 0·00001) (Fig. 1b).

Disease assessment in different tissue types was repeated on a subset of plants chosen for gas exchange measurement. ELISA tests were made directly after gas exchange measurement on 21 July (92 DAP) and 11 August (116 DAP) 2003. At the time of gas exchange measurement, plants were categorized according to previous ELISA results assessed on 27 June 2003 (70 DAP). However, in the time period between 27 June and 21 July, some category 1 plants were infected. When these infected category 1 plants were measured for gas exchange on 21 July and 11 August, they did not have any visible TSWV symptoms (and no previous history of positive

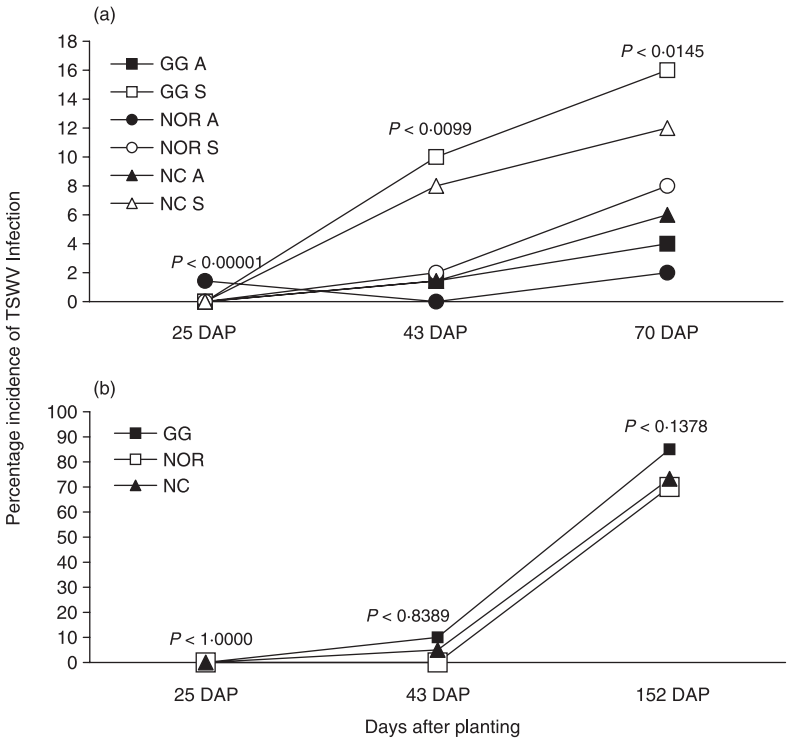


Figure 1 Incidence of *Tomato spotted wilt virus* (TSWV) (%) in leaves and roots of three peanut cultivars (GG, Georgia Green; NOR, ANorden; NC, NCV11) evaluated visually and by ELISA. Leaves were measured during early to mid-season infection at 25, 43 and 70 days after planting (DAP), and roots during early season (25 and 43 DAP) and at final harvest (152 DAP). Leaves include those with visual symptoms (S) and those without (A). *P*-values refer to significant differences within a single harvest data for leaves with and without symptoms across cultivars (a); and for roots among cultivars (b).

ELISA tests), even though they showed positive ELISA tests after gas exchange. When measuring disease incidence in this subset of plants chosen for physiological measurement, there were no significant differences in incidence of disease among peanut cultivars for any tissue type (leaves, pegs, roots or kernels) or between either the 21 July or 11 August harvest date. Overall disease incidence was significantly lower in leaves without symptoms than in those showing symptoms at both harvest dates (92 DAP: Wald chi-squared = 15·2, *P*-value = 0·0001; 116 DAP: Wald chi-squared = 12·0, *P*-value = 0·0005) (Fig. 2a and b). Disease incidence in below-ground structures showed significant differences among plant categories on 21 July (92 DAP) for pegs (Wald chi-squared = 10·7, *P*-value = 0·0048), roots (Wald chi-squared = 10·5, *P*-value = 0·0052) and kernels (Wald chi-squared = 9·4, *P*-value = 0·0089). On 11 August (116 DAP), this same pattern was seen, but only in pegs (Wald chi-squared = 7·2, *P*-value = 0·0270) (Fig. 3a–c).

## Physiology of TSWV-affected plants

Differences among cultivars in gas exchange analyses were only apparent on the 11 August (116 DAP) harvest, with the exception of water-use efficiency at the first harvest, 27 June (70 DAP) (Table 1). At this first harvest date, cv. ANorden had significantly higher water-use efficiency than the cv. NC-V11, but was not different from cv. Georgia Green (Fig. 4). On this same date, there were no other effects on gas exchange detected, as leaves with and without symptoms had similar gas exchange characteristics

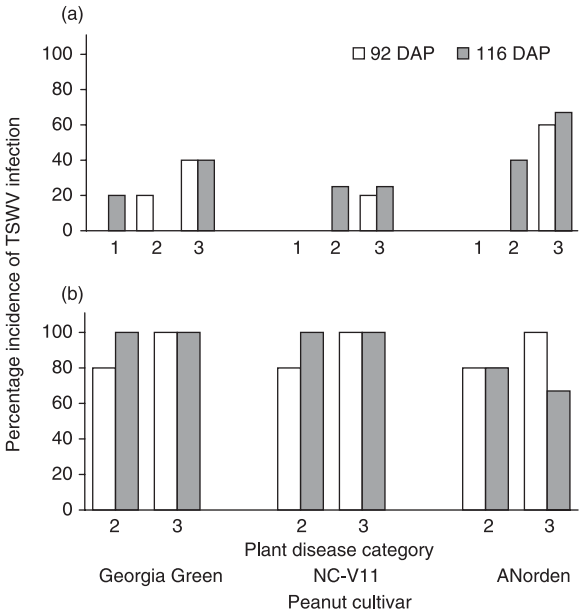


Figure 2 Incidence and severity of *Tomato spotted wilt virus* (TSWV) infection (%) in leaves of three peanut cultivars (Georgia Green, NC-V11 and ANorden) at two harvest periods 92 and 116 days after planting (DAP). Both leaves without (a) and with visual symptoms (b) were measured. Category 1, plants with no visual TSWV symptoms and not previously positive for TSWV by ELISA; category 2, plants < 50% of leaves with visual symptoms and previously positive for TSWV by ELISA; category 3, plants > 50% of leaves with visual symptoms and previously positive for TSWV by ELISA.

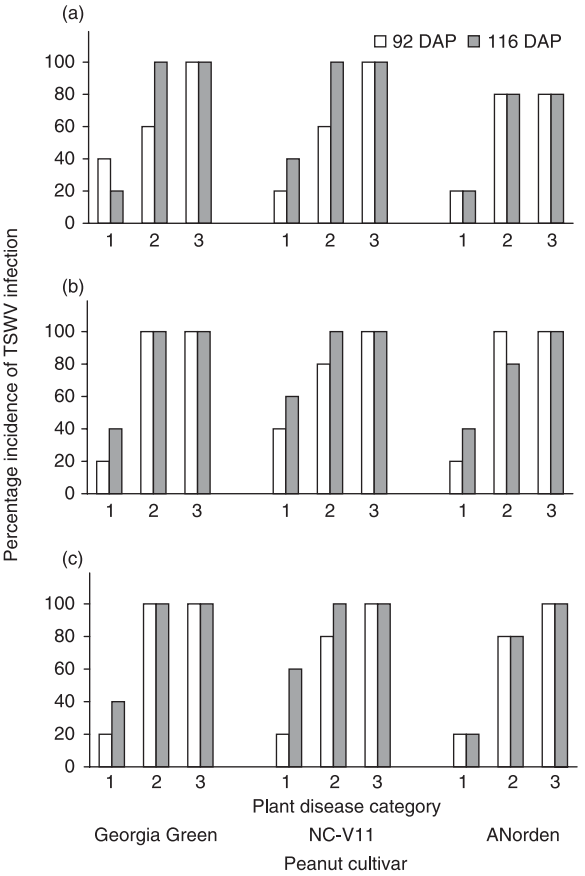


Figure 3 Incidence and severity of *Tomato spotted wilt virus* (TSWV) infection (%) for below-ground plant organs of three peanut cultivars (Georgia Green, NC-V11 and ANorden) at two harvest periods, 92 and 116 days after planting (DAP). Tissue includes pegs (a), peanut pods (including kernels) (b) and roots (c). Category 1, plants with no visual TSWV symptoms and not previously positive for TSWV by ELISA; category 2, plants having < 50% of leaves with visual symptoms and previously positive for TSWV by ELISA; category 3, plants having > 50% of leaves with visual symptoms and previously positive for TSWV by ELISA.

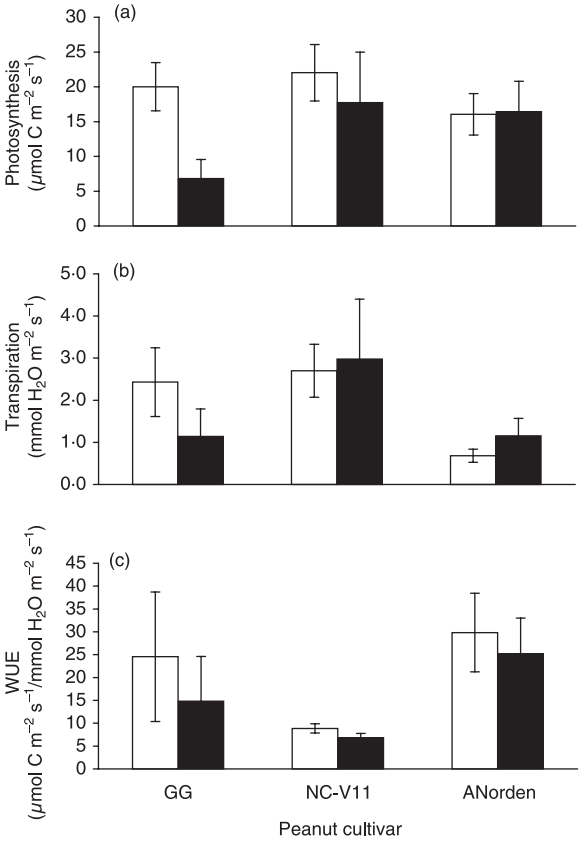


Figure 4 Photosynthesis (a), transpiration (b) and water-use efficiency (WUE) (c) in leaves with (black bars) and without symptoms (white bars) of *Tomato spotted wilt virus* (TSWV) in three peanut cultivars, Georgia Green (GG), NC-V11 and ANorden, at 70 days after planting (DAP). Error bars indicate standard error of the mean ($n$ = 36).

and there was no interaction between cultivar and leaf type (Table 1, Fig. 4).

At the two subsequent harvest dates, more effects on gas exchange were detected. At the middle harvest, 21 July (92 DAP), previously noninfected plants (category 1) had significantly higher photosynthetic rates than category 2- and 3-infected plants. In addition, leaves with symptoms had significantly lower photosynthetic rates and water-use efficiency than symptomless leaves (Table 1, Fig. 5). Water-use efficiency also showed an interaction between cultivar and disease category such that infected plants (category 2 and 3) of cv. NC-V11 had slightly higher water-use efficiency than category 1 plants, while the other cultivars had the opposite pattern for this trait. On 11 August (116 DAP), cultivars were significantly different from one another in both rates of photosynthesis and transpiration, with water-use efficiency showing borderline significance (Table 1). The cultivar ANorden had a significantly higher rate of photosynthesis than cv. Georgia Green and higher transpiration than cv. NC-V11. At this harvest, disease severity had a significant effect on photosynthesis and water-use efficiency, with category 1 plants exhibiting significantly higher photosynthesis than both category 2 and 3 plants, and higher water-use efficiency than the most infected category 3 plants (Fig. 5). Also, at this harvest date, leaves (across cultivars and plant categories) with symptoms had significantly lower photosynthetic levels than leaves without symptoms (Table 1, Fig. 5).

Gas exchange was significantly affected by TSWV infection. When gas exchange was compared in symptomless leaves alone, root infection significantly affected both photosynthesis and transpiration. At the 21 July harvest (92 DAP), root infection significantly decreased photosynthesis (d.f. = 1, $F$-ratio = 15·31, $P$-value = 0·0004) and transpiration (d.f. = 1, $F$-ratio = 6·74, $P$-value = 0·0133) regardless of cultivar; however, at the 11 August harvest (116 DAP), the effect was not significant even though the means remained numerically higher in the TSWV-negative plants (Fig. 6). The actual percentage decrease in gas

Table 1 Analysis of variance results of the effect of peanut cultivar, plant category and leaf type and their interactions on physiological effects of *Tomato spotted wilt virus* (TSWV) on cvs Georgia Green, NC-V11 and ANorden at three harvest dates, 70, 92 and 116 days after planting (DAP). At 92 and 116 DAP, plants were categorized according to severity of TSWV symptoms: category 1, plants with no visual TSWV symptoms and not previously positive for TSWV by ELISA; category 2, plants having < 50% of leaves with visual symptoms and previously positive for TSWV by ELISA; category 3, plants having > 50% of leaves with visual symptoms and previously positive for TSWV by ELISA. Leaves with and without symptoms were evaluated as separate leaf types. Numbers in bold are statistically significant values

| | Photosynthesis | | Transpiration | | Water-use efficiency | |
|---|---|---|---|---|---|---|
| | *F*-ratio | *P*-value | *F*-ratio | *P*-value | *F*-ratio | *P*-value |
| 70 DAP | | | | | | |
| Cultivar | 1·08 | 0·3509 | 3·01 | 0·0648 | **6·85** | **0·0038** |
| Leaf type | 2·51 | 0·1234 | 0·31 | 0·5810 | 0·84 | 0·3682 |
| Cultivar × leaf type | 1·23 | 0·3066 | 0·56 | 0·5784 | 0·01 | 0·9925 |
| 92 DAP | | | | | | |
| Cultivar | 0·17 | 0·8441 | 0·52 | 0·5964 | 0·13 | 0·8763 |
| Category | **6·27** | **0·0033** | 1·30 | 0·2784 | 2·49 | 0·0913 |
| Leaf type | **13·42** | **0·0005** | 0·93 | 0·3392 | **15·19** | **0·0002** |
| Cultivar × category | 0·85 | 0·4982 | 0·68 | 0·6071 | **2·84** | **0·0314** |
| Cultivar × leaf type | 0·57 | 0·5672 | 0·42 | 0·6591 | 0·61 | 0·5472 |
| 116 DAP | | | | | | |
| Cultivar | **5·85** | **0·0053** | **5·03** | **0·0103** | 3·08 | 0·0551 |
| Category | **8·88** | **0·0005** | 2·96 | 0·0608 | **4·30** | **0·0190** |
| Leaf type | **6·42** | **0·0146** | 2·15 | 0·1488 | 3·67 | 0·0613 |
| Cultivar × category | 0·44 | 0·7807 | 1·15 | 0·3418 | 0·29 | 0·8798 |
| Cultivar × leaf type | 0·38 | 0·6826 | 0·11 | 0·8920 | 1·56 | 0·2203 |

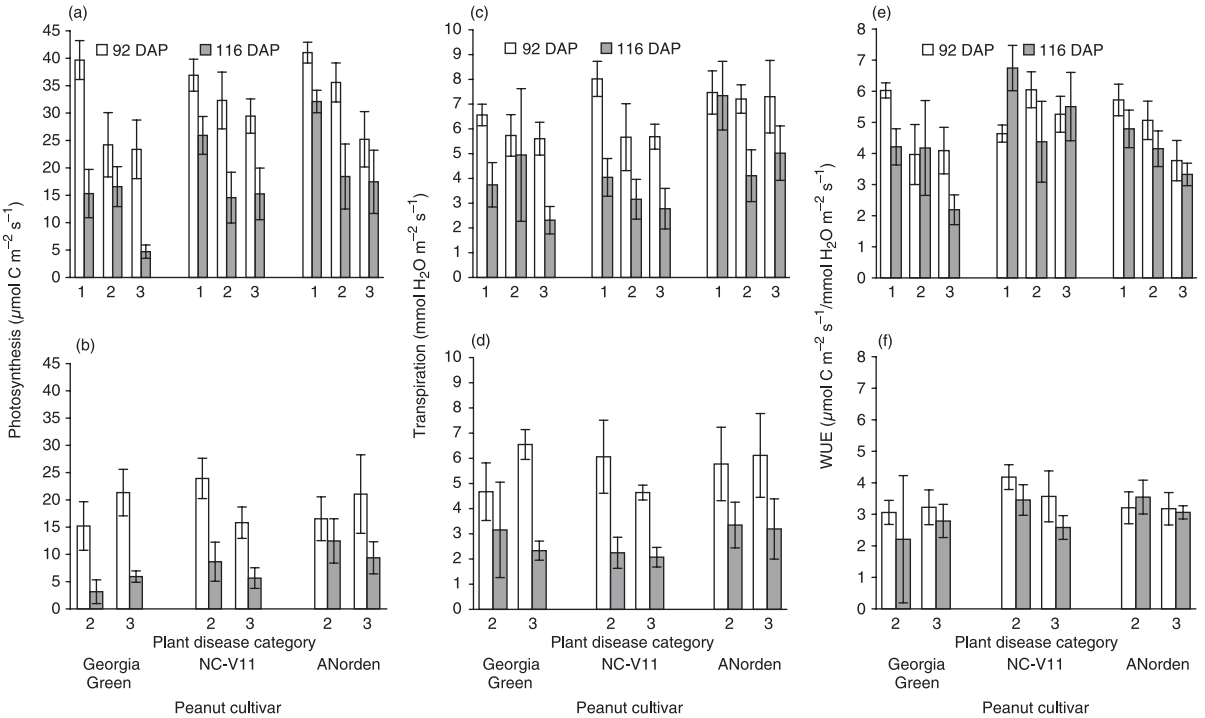


Figure 5 Photosynthesis, transpiration and water-use efficiency for three peanut cultivars (Georgia Green, NC-V11 and ANorden) at two harvest periods, 92 and 116 days after planting (DAP). Both leaves with (b, d, f) and without (a, c, e) visual *Tomato spotted wilt virus* (TSWV) symptoms were tested. Category 1, plants with no visual TSWV symptoms and not previously positive for TSWV by ELISA; category 2, plants having < 50% of leaves with visual symptoms and previously positive for TSWV by ELISA; category 3, plants having > 50% of leaves with visual symptoms and previously positive for TSWV by ELISA. Error bars indicate standard error of the mean ($n$ = 75).

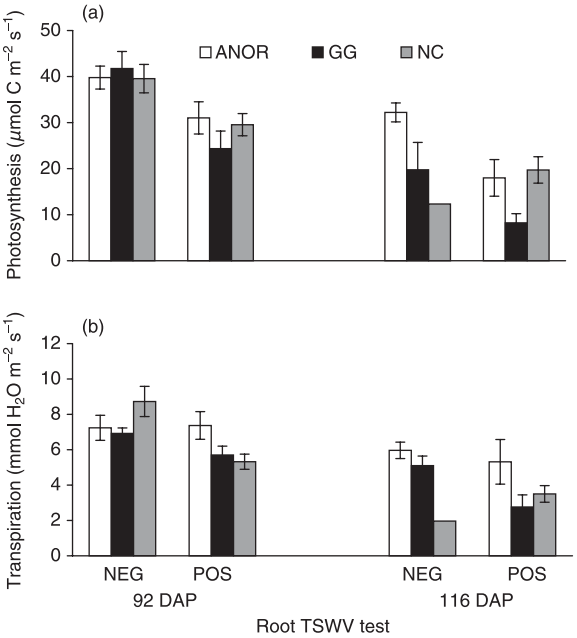


Figure 6 Photosynthesis (a) and transpiration (b) differences in symptomless leaves of *Tomato spotted wilt virus* (TSWV)-infected (POS) and noninfected (NEG) plants of three peanut cultivars, ANorden (ANOR), Georgia Green (GG) and NC-V11 (NC), at two harvest periods, 92 and 116 days after planting (DAP). Infection was determined by ELISA TSWV tests on root tissue at 152 DAP. Error bars indicate standard error of the mean ($n$ = 44).

exchange was quite large as a result of infection and was significantly different among cultivars for photosynthesis on 11 August and 21 July (92 DAP). The decrease in photosynthesis was 22% for cv. ANorden, 42% for cv. Georgia Green, and 25% for cv. NC-V11; while transpiration at this harvest date showed a 2% increase for ANorden, but 18 and 39% decreases for cvs Georgia Green and NC-V11, respectively. At the 11 August harvest, the decreases were very dramatic, with 44 and 58% decreases in photosynthesis for ANorden and Georgia Green, respectively, while transpiration was decreased 11% in ANorden and 46% in Georgia Green. At this last harvest, values for cv. NC-V11 may be biased since only one plant tested negative for TSWV (Fig. 6).

The other leaf physiological traits measured showed limited differences among cultivars, disease categories and leaf types. At the 21 July (92 DAP) harvest, only cv. Georgia Green showed differences for mean RWC (d.f. = 2, $F$-ratio = 3·3799, $P$-value = 0·0403), and differences for IRT surface temperature, SPAD chlorophyll content or specific leaf area at this date were only apparent between leaves with and without symptoms. SPAD chlorophyll content was greatest in symptomless leaves at both 92 and 116 DAP (d.f. = 1, $F$-ratio = 50·6, $P$-value = 0·0001; d.f. = 1, $F$-ratio = 12·3, $P$-value = 0·0010, respectively); while leaves with symptoms had higher specific leaf area at 92 DAP (d.f. = 1, $F$-ratio = 14·4, $P$-value = 0·0003).

### Yield

There were no significant differences among cultivars for yield (d.f. = 2, $F$-ratio = 0·11, $P$-value = 0·8956), but cv. NC-V11 had significantly lower percentage kernel weight (shelled kernel weight not including hull weight) than the other two cultivars (d.f. = 2, $F$-ratio = 23·25, $P$-value = 0·0015). Yields ranged from 2864 kg ha$^{-1}$ with 72% shell-out, 2762 kg ha$^{-1}$ with 77% shell-out, and 2852 kg ha$^{-1}$ with 78% shell-out, for cvs NC-V11, ANorden and Georgia Green, respectively.

## Discussion

The data in this study indicated that TSWV severely affects physiology of peanut plants. Photosynthesis, transpiration and water-use efficiency were significantly decreased in virus-affected plants, and the effect of these processes depended on severity of disease. There were differences, too, in the progress of TSWV through different peanut tissue types, with below-ground tissues exhibiting greater disease incidence than leaves.

Progress of TSWV in the monitored crops was slow at the beginning, with the virus being detected in both leaves and roots at 43 DAP. Higher levels of disease, particularly in the leaves, occurred later at 70 DAP. The rate of progress of TSWV has been reported to be dependent on the cultivar (Culbreath *et al*., 1992b), but in the present study, disease progression in both leaves and roots followed a somewhat linear pattern with no differences among cultivars. There was certainly evidence of early season infection but the somewhat exponential pattern of root infection was more indicative of late-season infection. In addition, the absence of physiological differences between leaves with and without symptoms at 70 DAP suggests TSWV affects plant physiology later in the season. Early season infection often causes stunting and eventual seedling death, while crops infected later on usually experience destruction of the root system and loss of yield (Lyerly *et al*., 2002). Most tagged plants survived the entire season, which supports a pattern of late-season infection with higher disease incidence/severity in roots. It appeared that infection progressed from below-ground to above-ground plant parts, but the process of TSWV translocation was not determined in this study. Mandal *et al*. (2002) found some evidence in certain peanut cultivars of the restriction of long-distance movement of TSWV through different parts of the plant, thereby blocking the systemic movement of the virus. While this characteristic may impart greater field resistance to TSWV, the present study has no evidence of differences in virus infection or progression among the peanut cultivars measured. TSWV has been found to be concentrated in bud terminals where thrips larvae feed, but appears to be high in young leaves as well (Kresta *et al*., 1995). In this study, leaf terminals were not measured; instead the second nodal (first fully expanded leaf) was measured and it is likely that TSWV levels were high in these newly formed tissues and therefore easily detected.

Virus-infected plants generally have reduced photosynthetic levels in comparison to their noninfected counterparts, primarily due to a reduction in photosystem II (Cabaleiro *et al*., 1999; Clover *et al*., 1999a; Arias *et al*., 2003; Bertamini *et al*., 2004). These reductions are often seen throughout the season. Naidu *et al*. (1984) found *Peanut green mosaic virus* decreased photosynthesis and chlorophyll content from early infection to establishment of systemic infection. Decreases in photosynthesis caused by TSWV reported here of between 22 and 42% in the first harvest, and as much as 58% in the last harvest, reflect values recorded in other studies. For example, Sampol *et al*. (2003) reported a decrease of almost 50%, caused by a decrease in carboxylation and possibly mesophyll conductance rather than stomatal conductance. In sugar beet and *Nicotiana benthamiana*, viral infection caused a 20–50% decrease in photosynthesis (Clover *et al*., 1999a; Swiech *et al*., 2001). In the present study, decreases in photosynthesis were of greater magnitude later in the season, perhaps reflecting the pattern of a more severe late-season infection, the imposed drought or compounding effects of plant age or senescence.

One of the most marked differences in photosynthesis was between leaves with and without symptoms and between plants in different disease severity categories. At 92 DAP, leaves without symptoms had significantly higher rates of photosynthesis than leaves with symptoms, since the latter are likely to have greater viral loads and, consequently, to have greater viral damage to the photosynthetic apparatus.

There was evidence that symptomless leaves from infected compared with symptomless leaves from noninfected plants were physiologically different. The existence of TSWV-infected leaves that remained symptomless has been previously documented. In a study evaluating *Arachis* spp. for resistance to TSWV, there were several instances of peanut plants infected with TSWV that exhibited no visible symptoms, while some plants recovered from initial infection and appeared normal (Lyerly *et al*., 2002). In another study, nearly 18% of symptomless leaf tissue (excluding terminals) was infected with TSWV (Kresta *et al*., 1995). However, results of the present study showed that the absence of visual symptoms does not mean that leaf physiology is not affected, since there was significant suppression in symptomless leaves of both photosynthesis and transpiration at 92 DAP, regardless of cultivar. There has been some limited evidence of a disparity between symptomless infected and noninfected leaves in other plant species. Cabaleiro *et al*. (1999) found that leafroll virus lowered photosynthesis in symptomless leaves of infected grapevine plants compared with leaves of noninfected plants. However, Arias *et al*. (2003) found no changes in $CO_2$ fixation, respiration or carbohydrate accumulation in infected sunflower plants until symptoms became evident, and alterations to gas exchange were not observed in symptomless leaves. These reports may reflect two different processes occurring in symptomless leaves of infected plants: one where the alterations to physiology are brought about by viral presence alone, so that the effect of viral infection on gas exchange is due to the build-up of virus in leaf tissue; or a second process where the physiological effects may be due to oxidative stress so that even leaves without high viral loads are physiologically suppressed (Arias *et al*., 2003). TSWV appears to cause the latter process in peanuts because this study quantified a level of photosynthesis suppression even in apparently nonstressed leaf tissue.

Reductions in photosynthesis due to viral infection appear to accompany decreases in transpiration, chlorosis and certain leaf morphological changes. In this study, transpiration was significantly suppressed due to viral infection. This result is similar to other studies where there were simultaneous decreases in photosynthesis and transpiration with viral infection (Clover *et al*., 1999b; Bertamini *et al*., 2004). In addition to gas exchange alterations, viral infection often causes chlorosis (Swiech *et al*., 2001; Arias *et al*., 2003; Bertamini *et al*., 2004). Peanut leaf chlorophyll content in this study was consistently lower in leaves with symptoms than in those without symptoms, irrespective of cultivars or plant disease categories, probably due to damage to leaf photosynthetic apparatus caused by TSWV infection. In addition, leaves with TSWV and expressing symptoms were thicker (lower SLA) than symptomless leaves, a result that agrees with Swiech *et al*. (2001), who found thicker leaves in virus-infected sugar beet plants because of a general enlargement of mesophyll cells. This cell enlargement may decrease photosynthesis by interfering with light interception and diffusion of $CO_2$ into the leaf (Swiech *et al*., 2001). Leaf water status did not appear to be greatly affected by TSWV infection, with only inherent differences among cultivars apparent.

In general, cultivars did not express real differences in TSWV infection, and physiological differences were seen only for cv. ANorden, which had significantly higher photosynthesis than cv. Georgia Green and higher transpiration than cv. NC-V11. The lack of large cultivar differences may be due to the similar TSWV susceptibility ratings for the three cultivars utilized in this study. Georgia Green and NC-V11 have been reported to have moderate levels of TSWV field resistance (Culbreath *et al*., 2000), but in comparison to more recent cultivar releases they are still considered susceptible (Mandal *et al*., 2002; Wells *et al*., 2002). Georgia Green has been known to suffer significant damage caused by TSWV in severe outbreaks of the virus (Wells *et al*., 2002). Cultivar NC-V11, when compared with other Virginia-type cultivars, ranks highly in TSWV resistance levels (Garcia *et al*., 2000). In other comparisons, NC-V11 has inconsistent resistance levels to TSWV with a range of resistance and susceptibility, but has often been found to have a similar level of TSWV resistance to cv. Georgia Green (Culbreath *et al*., 2000). ANorden is a newly released cultivar but is considered to have moderate TSWV resistance as well. Yield differences among cultivars were not evident in this study either, despite some differences in physiology. This result may be due to the limited plot size and plant perturbation associated with experimental measurements in this study,

whereas yield differences may be more likely in normal crop production. In addition, physiological differences were most apparent late in the season, indicating that infection may also have occurred late, and, in general, the later the infection occurs in the season, the lower the yield loss (Culbreath *et al*., 1992a; Garcia *et al*., 2000). Furthermore, Wells *et al*. (2002) found that only 13–33% of yield variation among cultivars in a given year could be explained by TSWV, so that yield loss was not always closely allied to incidence of the virus.

This study identified a possible TSWV tolerance mechanism that is physiologically based: the ability to maintain near-normal photosynthetic levels in symptomless tissue, even in the presence of viral infection. Lyerly *et al*. (2002) suggested that this type of suppressive mechanism, present in some peanut cultivars, was not resistance to TSWV *per se*. The ability to maintain high physiological function in the presence of TSWV may be a more important resistance or tolerance mechanism than actual avoidance of infection because of the near omnipresence of TSWV in USA peanut-producing regions. A survey of fields in Georgia in 1989 found evidence of TSWV symptoms in every field observed (Culbreath *et al*., 1992b) and a year later the virus was present in 100% of fields surveyed in 10 counties in Georgia (Camann *et al*., 1995). The information presented in the current study is important because a better understanding of the mechanisms behind the viral impact on host plant physiology can lead to the development of improved cultivars that either resist viral infection or can better tolerate infection by experiencing less severe symptoms (Balachandran *et al*., 1997).

## Acknowledgements

We thank Kathy Gray, Sam Hilton, Milbra Schweikert, Valerie Orner, Eva Whitehead, Latoya Rucker, William Pearce and Larry Powell for making this laboratory and field work possible. We thank the National Peanut Board for financial assistance for this project.

## References

Agrios GN, 1997. *Plant Pathology*, 3rd edn. New York, NY, USA: Academic Press.

Arias MC, Lenardon S, Taleisnik E, 2003. Carbon metabolism alterations in sunflower plants infected with the *Sunflower chlorotic mottle virus*. *Journal of Phytopathology* **151**, 267–73.

Armstrong JS, Kraemer GC, Mitchell FL, 2001. Thrips species associated with west Texas peanut. *Southwestern Entomologist* **26**, 345–52.

Ayres PG, 1992. Plants versus pests and pathogens: an old story but the same story?. In: Ayres PG, ed. *Pests and Pathogens: Plant Response to Foliar Attack*. Oxford, UK: Bios Scientific Publishers, 1–10.

Balachandran S, Hurry VM, Kelley SE, Osmond CB, Robinson SA, Rohozinski J, Seaton GGR, Sims DA, 1997. Concepts of plant biotic stress. Some insights into stress physiology of virus-infected plants, from the perspective of photosynthesis. *Physiologia Plantarum* **100**, 203–13.

Bertamini M, Muthuchelian K, Nedunchezhian N, 2004. Effect of grapevine leafroll on the photosynthesis of field grown grapevine plants (*Vitis vinifera* L. cv. Lagrein). *Journal of Phytopathology* **152**, 145–52.

Brittlebank CC, 1919. Tomato diseases. *Journal of Agriculture, Victoria* **27**, 231–5.

Cabaleiro C, Segura A, García-Berrios JJ, 1999. Effects of grapevine leafroll-associated virus 3 on the physiology and must of *Vitis vinifera* L. cv. Albarino following contamination in the field. *American Journal of Enology and Viticulture* **50**, 40–4.

Camann MA, Culbreath AK, Pickering J, Todd JW, Demski JW, 1995. Spatial and temporal patterns of spotted wilt epidemics in peanut. *Phytopathology* **85**, 879–85.

Clover GRG, Azam-Ali SN, Jaggard KW, Smith HG, 1999a. The effects of beet yellows virus on the growth and physiology of sugar beet (*Beta vulgaris*). *Plant Pathology* **48**, 129–38.

Clover GRG, Smith HG, Azam-Ali SN, Jaggard KW, 1999b. The effects of drought on sugar beet growth in isolation and in combination with beet yellows virus infection. *Journal of Agricultural Science* **133**, 251–61.

Culbreath AK, Todd JW, Demski JW, 1992a. Productivity of Florunner peanut infected with tomato spotted wilt virus. *Peanut Science* **19**, 11–4.

Culbreath AK, Todd JW, Demski JW, Chamberlin JR, 1992b. Disease progress of spotted wilt in peanut cultivars Florunner and Southern Runner. *Phytopathology* **82**, 766–71.

Culbreath AK, Todd JW, Gorbet DW, Brown SL, Baldwin JA, Pappu HR, Holbrook CC, Shokes FM, 1999. Response of early, medium, and late maturing peanut breeding lines to field epidemics of tomato spotted wilt. *Peanut Science* **26**, 100–6.

Culbreath AK, Todd JW, Gorbet DW, Brown SL, Baldwin J, Pappu HR, Shokes FM, 2000. Reaction of peanut cultivars to spotted wilt. *Peanut Science* **27**, 35–9.

Ehleringer JR, Osmond CB, 1989. Stable isotopes. In: Pearcy RW, Ehleringer JR, Mooney HA, Rundel PW, eds. *Plant Physiological Ecology*. London, UK: Chapman & Hall, 281–300.

Garcia LE, Brandenburg RL, Bailey JE, 2000. Incidence of *Tomato spotted wilt virus* (Bunyaviridae) and tobacco thrips in Virginia-type peanuts in North Carolina. *Plant Disease* **84**, 459–64.

Goldbach R, Peters D, 1994. Possible causes of the emergence of Tospovirus diseases. *Seminars of Virology* **5**, 113–20.

Kresta KK, Mitchell FL, Smith JW Jr, 1995. Survey by ELISA of thrips (Thysanoptera: Thripidae) vectored tomato spotted wilt virus distribution in foliage and flowers of field-infected peanut. *Peanut Science* **22**, 141–9.

Lyerly JH, Stalker HT, Moyer JW, Hoffman K, 2002. Evaluation of *Arachis* species for resistance to *Tomato spotted wilt virus*. *Peanut Science* **29**, 79–84.

Mandal B, Pappu HR, Culbreath AK, Holbrook CC, Gorbet DW, Todd JW, 2002. Differential response of selected peanut (*Arachis hypogaea*) genotypes to mechanical inoculation by *Tomato spotted wilt virus*. *Plant Disease* **86**, 939–44.

Naidu RA, Krishnan M, Ramanujam P, Gnanam A, Nayudu MV, 1984. Studies on peanut green mosaic virus infected peanut (*Arachis hypogaea* L.) leaves. I. Photosynthesis and photochemical reactions. *Physiological Plant Pathology* **25**, 181–90.

Pappu SS, Pappu HR, Culbreath AK, Todd JW, 1999. Localization of *Tomato spotted wilt virus* (Genus *Tospovirus*,

Family *Bunyaviridae*) in peanut pods. *Peanut Science* **26**, 98–100.

Riley D, 2004. Thrips and tomato spotted wilt management in tomato – a cost effective IPM program. Georgia IPM. On-line *http://www.gaipm.org/vegetable/thripstswv.html* [accessed 11 March 2005].

Ryšlavá H, Muller K, Semorádová Š, Synková H, Čeøovská N, 2003. Photosynthesis and activity of phosphoenolpyruvate carboxylase in *Nicotiana tabacum* L. leaves infected by *Potato virus A* and *Potato virus Y*. *Photosynthetica* **41**, 357–63.

Sampol B, Bota J, Riera D, Medrano H, Flexas J, 2003. Analysis of the virus-induced inhibition of photosynthesis in malmsey grapevines. *New Phytologist* **160**, 403–12.

SAS, 1997. *JMP Statistical Discovery Software*. Cary, NC, USA: SAS Institute Inc.

Shalitin D, Wolf S, 2000. Cucumber mosaic virus infection affects sugar transport in melon plants. *Plant Physiology* **123**, 597–604.

Stewart JW, Cole C, Lummus P, 1989. Winter survey of thrips (Thysanoptera: Thripidae) from certain suspected and confirmed hosts of tomato spotted wilt virus in south Texas. *Journal of Entomological Science* **24**, 392–401.

Swiech R, Browning S, Molsen D, Stenger DC, Holbrook GP, 2001. Photosynthetic responses of sugar beet and *Nicotiana benthamiana* Domin. infected with beet curly top virus. *Physiological and Molecular Plant Pathology* **58**, 43–52.

Todd JW, Culbreath AK, Chamberlin JR, Beshear RJ, Mullinix BG, 1995. Colonization and population dynamics of thrips in peanuts in the southern United States. In: Parker BL, Skinner M, Lewis T, eds. *Thrips Biology and Management*. New York, USA: Plenum Press, 453–60.

Wells ML, Culbreath AK, Todd JW, Brown SL, Gorbet DW, 2002. A regression approach for comparing field resistance of peanut cultivars to tomato spotted wilt tospovirus. *Crop Protection* **21**, 467–74.

Zaitlin M, Hull R, 1987. Plant virus–host interactions. *Annual Review of Plant Physiology* **38**, 291–315.